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ISOLATION, IDENTIFICATION AND SYNTHESIS OF THE SEX PHEROMONE OF THE CITRUS MEALYBUG, PLANOCOCCUS CITRI (RISSO)

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Abstract: The sex attractant pheromone of the citrus mealybug, Planococcus citri (Risso), has been identified as (1R-cis)-(+)-2,2-dimethyl-3-(1-methylethenyl)cyclobutanemethanol acetate (VI).

The citrus mealybug, Planococcus citri (Risso), a cosmopolitan insect found on numerous hosts, is an economic problem in citrus groves and greenhouses in California and Texas[1-3]. The insects characteristically aggregate in compact groups, and their feeding results in a general withdrawal of sap which, at the time of blossoming, may cause flowers, newly set fruit, and young foliage to drop. Additional damage is caused by the honeydew deposits that cause smutting, discoloration of leaves and fruit, retardation of growth in the fruit, and subsequent dropping of mature foliage. The females release a sex pheromone that has now been isolated, identified and synthesized and shown to be highly attractive to males in field tests.

The pheromone was trapped on Porapak Q[4] from an air stream[5] passed over large numbers of adult, laboratory-reared, virgin female insects feeding on potato sprouts. Initially, the females used were isolated by hand sorting but later in the program, the first-molt insects were dipped in a solution of the juvenoid triprene (S-ethyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienethioate); this treatment allowed the females to develop normally but prevented male metamorphosis[6]. The pentane wash of the Porapak was chromatographed on silica gel and then on 20% $AgNO_3$ on Florisil; the hexane-ether fractions were monitored by laboratory bioassay[5]. The active material was eluted as a single compound as shown by capillary gas chromatography (GC) on a non-polar column.

A GC reaction procedure whereby chemical reagents were used for subtraction of compounds containing specific functional groups[7] showed that the pheromone was not an alcohol, aldehyde or unhindered ketone. The presence of an acetate ester function was demonstrated by hydrolysis with alcoholic KOH and regeneration of the pheromone by acetylation. The volatiles trapped on the Porapak Q contained lesser amounts of the corresponding alcohol than of the acetate. Chemical ionization (CI) mass spectrometry (MS) showed that the molecular ion (M^+) of the pheromone was m/e 196, $C_{12}H_{20}O_2$. The electron impact (EI) spectrum showed losses of $M^+-C_5H_8$ at m/e 128(4.9%) and of $M^+-(CH_3COOH+CH_3)$ at m/e 121(4.4%); the base peak was C_5H_8 at m/e 68. The spectrum of the

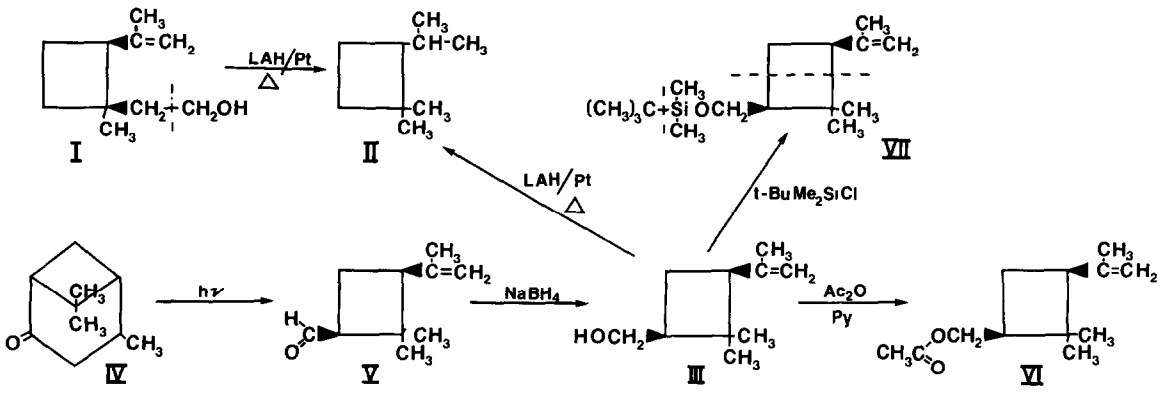
corresponding alcohol gave M^+ at m/e 154(0.4%), M^+-CH_3 at m/e 139(5.6%), M^+-CH_2OH at m/e 123(12.4%), $M^+-(H_2O + CH_3)$ at m/e 121(11.5%), and C_4H_7O at m/e 71(100%).

GC retention indices[8] of the acetate and the corresponding alcohol were 1280 and 1174, respectively, on a non-polar OV-1 column and 1932 and 2045 on a polar Silar 10C column. These values, in comparison with those of terpenes of known structure, suggested that the acetate group was primary. This was confirmed by reaction of the alcohol with trityl chloride; secondary and tertiary alcohols do not react under the same conditions. In addition, CrO_3-pyridine oxidation gave the corresponding aldehyde, as shown by CI-MS (M^+ at m/e 153 and M^+-H_2O at m/e 135).

The infrared spectrum of the pheromone showed absorption at 1645 and 888 cm^{-1} ($R^1R^2C=CH_2$). Ozonolysis[9] gave a single GC peak whose EI mass spectrum showed M^+ at m/e 198 and the base peak at m/e 43; this compound reacted with NAOH + I_2 in ethanol (iodoform test). These data suggested that the ozonolysis product had a CH_3CO group derived by cleavage of the $=CH_2$ of an isopropenyl group (i.e. $R^2=CH_3$). Since only 1 double bond was indicated, a monocyclic structure was required to satisfy the molecular formula, $C_{10}H_{17}O_2CCH_3$.

The identity of the carbon skeleton was obtained by a reaction-GC procedure[10] wherein microgram quantities of the pheromone alcohol and authentic terpene-type alcohols were reduced with $LiAlH_4$ + 5% Pt on Al_2O_3 at 250°C. This procedure reductively cleaves $-CH_2OH$ from primary alcohols and -OH from secondary and tertiary alcohols as well as saturates the double bonds. The mass spectrum of the product, 1,1-dimethyl-2-(1-methylethyl)cyclobutane(II), obtained from the pheromone alcohol (III) was identical with that of the paraffin derived from authentic 1-methyl-cis-2-(1-methylethenyl)cyclobutaneethanol[11] (I) and showed fragments at m/e 111(2.7%), 98(16%), 83(45%) and 56(100%).

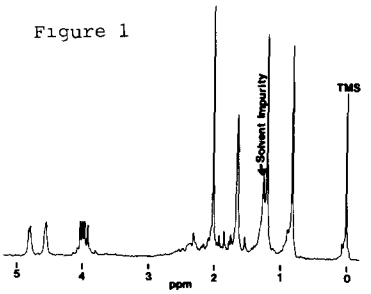
Scheme I



To establish the position of the $-CH_2OAc$ in the pheromone, a few micrograms of the alcohol were derivatized with t-butylchlorodimethylsilane. Characteristic fragments in the mass spectrum of the derivative (VII) at m/e 199 ($C_5H_8OSiMe_2Bu$) and m/e 143 ($C_5H_9OSiMe_2$) suggested structure III,

which would yield C_5 fragments on splitting of the cyclobutane ring. Other positions of $-CH_2OSiMe_2Bu$ on the ring would not be expected to yield these fragments nor be consistent with the NMR spectrum[12] (Figure 1) of the pheromone (VI), which showed overlapping doublets at 3.9-4.1 ppm due to the diastereotopic H's of $OCH_2\dot{C}H-$. Singlets were assigned as: 0.83 and 1.12 (gem-dimethyl[13]), 1.65 ($CH_3\dot{C}=CH_2$) 2.02 ($CH_3\dot{C}=O$) and 4.55 and 4.80 ($=CH_2$) ppm. On this evidence, structure VI was proposed for the pheromone. A cis or trans configuration could not be assigned on the basis of these data but the specific rotation $[\alpha]^{25}_{3130}$ was found to be +168°[12].

Figure 1



The cis isomer of the pheromone (VI) was synthesized as shown in Scheme 1. Photolysis of cis-(+)-verbanone[14] (IV) gave cis-(+)-2,2-dimethyl-3-(1-methylethenyl)cyclobutanal[15] (V), which was then reduced to the alcohol (III) with $NaBH_4$. Acetylation and then purification by HPLC (20% $AgNO_3$ on 5 μm Spherisorb) gave VI with bp 110-111°/18 mm and $[\alpha]^{25}_{3130}$ + 148°. The NMR spectrum of this synthetic cis-(+)-enantiomer matched that of the natural material, and the mass spectra and GC retention indices of the 2 compounds and their derivatives were also identical. The cis-(-) enantiomer of VI ($[\alpha]^{25}_{3130}$ -130°) was prepared in the same manner from cis-(-)-verbanone. The trans isomer was obtained in 25% yield by oxidation of V, esterification of the resulting acid, and equilibration of the methyl ester with NaOMe in MeOH for 24 hr[16]; reduction of the ester with $LiAlH_4$ followed by acetylation of the alcohol gave the trans isomer of VI. Alternatively, this isomer could be obtained by the equilibration[17] of p-chloroaniline Schiff's base of aldehyde V and subsequent regeneration of the aldehyde followed by reduction and acetylation. Capillary GC on a 60 m SE-52 column gave baseline resolution of cis and trans VI (retention times 19.62 and 20.44 min, respectively); the natural pheromone showed a single peak at the retention time of the cis isomer. The NMR spectrum of the trans compound (purified by preparative GC) showed some differences from that of the cis isomer; gem-dimethyl signals were at 0.97 and 1.11 ppm and the OCH_2 multiplet at 4.1-4.3 ppm.

Since the stereochemistry of the precursor trans-(+)-verbenol acetate was established as R for the asymmetric carbons of the cyclobutane ring[18], the pheromone must be assigned the (1R) configuration as (1R-cis)-(+)-2,2-dimethyl-3-(1-methylethenyl)cyclobutanemethanol acetate (VI).

The biological activity of the natural pheromone and the synthetic enantiomers was assessed in both laboratory[5] and field tests (Table 1). The (+) enantiomer of VI is as attractive to citrus mealybug males as the natural material; the (-) enantiomer, the trans isomer, and the alcohol precursor are at least 10-fold less active. Since the cis-(-) acetate was of lesser optical purity than the cis-(+) acetate, the activity shown by the former (Table 1) may be caused by contamination with the latter.

Table 1

Lure Source	No. landings[a] with lure		No. males trapped[b] with lure		
	1 μg	0.1 μg	10 μg	1 μg	0.1 μg
Natural pheromone	635	191	413	178	32
Syn. cis-(+)-(VI)	978	358	523	152	68
Syn. cis-(-)-(VI)	112	62	124	26	4
Syn. trans-(VI)			0		
Syn. cis-(+)-(III)			2		
40 female insects		2403		202	
Blank		0		0	

a) Laboratory bioassay; total of 4 replicates; analytical paper dispenser; 12 mm diam. circles.
b) Field bioassay in Riverside, CA; total of 5 replicate traps; rubber septum dispenser.

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